THE JUVENILE HORMONE. III. ITS ACCUMULATION AND STORAGE IN THE ABDOMENS OF CERTAIN MALE MOTHS

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In the previous papers of this series (Williams, 1959, 1961) a simple and highly selective test for the juvenile hormone was described in terms of its ability to suppress the transformation of pupae into adult moths. By the use of this so-called "pupal assay" the role of the juvenile hormone was investigated during successive stages of the metamorphosis of the Cecropia silkworm.

A by-product of these studies was a finding which one could scarcely have predicted by any rational process; namely, that the abdomens of male Cecropia and Cynthia moths contain a cache of juvenile hormone. The object of the present communication is to document this fact in experiments performed on five species of wild silkworms—Cecropia, Cynthia, Polyphemus, Pernyi, and Orizaba.

METHODS

In addition to methods previously described (Williams, 1959, 1961), the following special procedures were utilized:

1. *Parabiosis between pupae and headless moths*

The moth was deeply anesthetized with carbon dioxide. The antennae and the fore and midlegs were excised and melted wax was applied with a drawing-pen to cover the entire head and prothorax. Then, with a single transverse cut, the head was removed to leave a collar of wax at the anterior open end of the prothorax. Crystals of an equal-part mixture of phenylthiourea and streptomycin sulfate were placed in the wound along with enough Ringer's solution to fill the cavity.

The pupal partner was deeply anesthetized with carbon dioxide and a disc of integument, about 4 mm. in diameter, was cut from the mesothoracic tergum. The underlying epidermis was trimmed away with microscissors, care being taken to avoid any damage to the aorta which extends beneath the midline at this point. Melted wax was applied to the integument around the margin of the wound. Crystals of phenylthiourea and streptomycin were placed in the wound and the cavity was filled with a few drops of Ringer's solution.

The two animals were oriented in a cradle of plasticene and the wax-coated openings were brought into juxtaposition. The pupal abdomen was compressed until the pupal blood filled the narrow opening between the animals, all air being thereby displaced. The junction was then sealed with melted wax to yield a parabiotic preparation such as shown in Figure 1.

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The preparation was then removed from the anesthesia funnel and stored at 25° C. Under this condition, the moth commonly initiated energetic flapping of its wings. In order to prohibit this activity, the wings were placed between the jaws of a spring-loaded clothes-pin.

2. *Parabiosis between pupae and adult abdomens*

Melted wax was applied to the first abdominal segment of an anesthetized moth. With a single transverse scissor-cut the entire abdomen was detached from the thorax. The wax-coated edges of the wound were spread apart and the glistening air-filled crop was grasped with forceps and removed. Crystals of the phenylthiourea-streptomycin mixture were placed in the wound along with a few drops of Ringer's solution. The adult abdomen was then joined in parabiosis with a pupal partner, as described above. A preparation of this type is shown in Figure 3.

TABLE I

Parabiosis between diapausing Cecropia pupae and headless moths

Species of moth		Sex of pupal partner	Number of preparations	Results
Polyphemus	♀	♀	2	Prolonged survival* but no development
	♀	♂	2	Prolonged survival but no development
	♂	♀	2	Prolonged survival but no development
	♂	♂	2	Prolonged survival but no development
Pernyi	♀	♀	2	Prolonged survival but no development
	♀	♂	2	Prolonged survival but no development
	♂	♀	2	Prolonged survival but no development
	♂	♂	2	Prolonged survival but no development
Cecropia	♀	♀	2	Prolonged survival but no development
	♀	♂	2	Prolonged survival but no development
	♂	♀	5	Prolonged survival but no development (2) Pupae developed into moths retaining many pupal characters (3)
	♂	♂	4	Prolonged survival but no development (3) Pupa developed into moth retaining many pupal characters (1)

* Moths survived for up to 10 weeks; pupae survived up to 6 months.

Results

1. *Parabiosis between diapausing Cecropia pupae and headless moths*

Thirty-five diapausing Cecropia pupae were joined in parabiosis with headless Polyphemus, Pernyi, and Cecropia moths. Six of the preparations died within a week at 25° C. and were discarded. The behavior of the 29 viable preparations is outlined in Table I.

There are three points of interest in this table. The first is the spectacular prolongation of life in moths joined to pupal partners. The second point of interest is the lack of any developmental response in the vast majority of preparations. But the most surprising finding of all is the fact that 4 of 9 diapausing pupae initiated development when joined to headless male Cecropia moths; moreover, in each of these cases the pupa developed and molted into an adult which preserved numerous

Table II

Parabiosis between previously chilled pupae and headless moths

Species of moth	Species of pupal partners	Number of preparations	Effects on pupal partner
♀ Polyphemus	♂ or ♀ Polyphemus	4	Formed normal moths
♂ Polyphemus	♂ or ♀ Polyphemus	4	Formed normal moths
	♂ or ♀ Cecropia	4	Formed normal moths
♀ Cecropia	♂ or ♀ Polyphemus	4	Formed normal moths
	♂ or ♀ Cecropia	4	3 formed normal moths 1 formed moth retaining a patch of pupal cuticle on thoracic tergum
♂ Cecropia	♂ or ♀ Polyphemus	2	2 developed into moths retaining many pupal characters
	♂ or ♀ Cecropia	8	8 developed into moths retaining many pupal characters

pupal characteristics throughout head, thorax, and abdomen. These four pupae, in short, behaved as if they had been implanted with active corpora allata (Williams 1952a, 1959, 1961).

2. *Parabiosis between previously chilled pupae and headless moths*

The experiments now under consideration differed from the preceding in that the pupal partners possessed endocrinologically active brains and were therefore able to initiate adult development within a few days after being placed at room temperature.

Forty preparations were assembled, of which ten soon died and were discarded. Table II summarizes the several types of experiments that were performed. In each of the 30 viable preparations the pupal partner initiated adult development within ten days at 25° C. Attention is directed to the effects of the parabiosis on the course of this development.

When the headless partner was a male or female Polyphemus moth, the pupa

FIGURE 1. A headless male Cecropia moth is here joined in parabiosis with a previously chilled pupa of the Polyphemus silkworm.

FIGURE 2. After three weeks at 25° C. the pupa has molted to form a second pupal stage showing only traces of adult characters. The old pupal cuticle has been removed.

FIGURE 3. The abdomen of a male Cecropia moth is joined in parabiosis with a previously chilled Cecropia pupa.

FIGURE 4. After three weeks at 25° C. the pupa has molted to form a second pupal stage showing only traces of adult characters. The old pupal cuticle has been removed. The adult abdomen has molted its adult cuticle which is here shown partially peeled away.

underwent normal adult development. The same was true in 7 of 8 preparations in which the headless partner was a female Cecropia moth. But in all ten preparations in which the headless partner was a male Cecropia moth, the pupa metamorphosed, not into a normal moth, but into a creature which retained large areas of pupal cuticle (Fig. 2). It seems necessary to conclude that a male Cecropia moth, though headless and without any corpora allata, can somehow favor the release of juvenile hormone within the parabiotic preparation.

In the analysis of these experiments we have centered attention on the effects of the parabiosis on the pupal partner. But, what about the developmental response of the other half of the combination—the headless moth? For present purposes suffice it to say that in about a third of the preparations the developmental reaction of the pupa spread to the adult partner and caused the latter to molt. The molt extended over both the thorax and abdomen, but never included the wings. The old adult cuticle was detached from the underlying epidermis and replaced by a smooth new cuticle which was of adult type, except for the generalized absence of scales or hairs.

3. *Parabiosis between previously chilled pupae and moth thoraces and abdomens*

Each of a series of ten male Cecropia moths was beheaded and subdivided into thorax and abdomen. The two parts were then joined in parabiosis with previously chilled Polyphemus pupae and placed at 25° C.

Within the following month all the surviving pupae which had been joined to adult thoraces emerged as normal moths. By contrast, all of the surviving pupae which had been joined to adult abdomens developed into moths retaining prominent pupal characteristics. This result demonstrated that juvenile hormone activity was associated with the abdomen of male Cecropia moths and that the thorax was inert in this respect.

4. *Juvenile hormone activity in relation to the species and sex of the moth abdomen*

Table III summarizes a large series of experiments which were performed to test the abdomens of male and female saturniid moths belonging to five different species. A total of 110 parabiotic preparations was established, of which 83 were viable.

Here again we see that the abdomens of male Cecropia moths always provoked strong positive tests for juvenile hormone. The same result was obtained for the abdomens of male Cynthia moths. The only other species to give a positive test was one of six abdomens of male Orizaba moths. The abdomens of both male and female Polyphemus moths were inactive; the same was true for the closely related Pernyi moth and for most males and all females of Orizaba.

Juvenile hormone activity in these adult insects is therefore a species-specific characteristic which is seen most prominently in Cecropia and Cynthia moths. Even here, it is a sex-linked characteristic being routinely encountered in males and only rarely, if at all, in female moths.

Though the outcome is strikingly dependent on the species and sex of the adult component in the parabiotic preparations, the result is seemingly independent of the species or sex of the pupal partner. This fact is emphasized in Table III where we see that male Cecropia abdomens gave uniformly positive tests when

TABLE III

Parabiosis between previously chilled pupal and moth abdomens

Adult abdomen	Species of pupal partner	Number of preparations	Effects on pupal partner
♀ Polyphemus	♂ or ♀ Polyphemus	4	Formed normal moths
♂ Polyphemus	♂ or ♀ Polyphemus	6	Formed normal moths
♀ Pernyi	♂ or ♀ Polyphemus	2	Formed normal moths
♂ Pernyi	♂ or ♀ Polyphemus	4	Formed normal moths
♀ Orizaba	♂ or ♀ Polyphemus	3	Formed normal moths
♂ Orizaba	♂ or ♀ Polyphemus	6	5 formed normal moths 1 formed moth retaining a patch of pupal cuticle on thoracic tergum
♀ Cecropia	♂ or ♀ Polyphemus	3	Formed normal moths
	♀ Cecropia	5	Formed normal moths
♂ Cecropia	♂ or ♀ Polyphemus	21	Developed into moths retaining many pupal characters
	♀ Cecropia	13	Developed into moths retaining many pupal characters
	♂ Cecropia	4	Developed into moths retaining many pupal characters
♀ Cynthia	♂ or ♀ Polyphemus	6	Formed normal adults
♂ Cynthia	♂ or ♀ Polyphemus	6	Developed into moths retaining many pupal characters

joined to pupae of male Cecropia, female Cecropia, male Polyphemus, or female Polyphemus.

In about a third of the preparations the adult abdomen underwent a molt in synchrony with the development of the pupal partner (Figs. 4 and 7). This phenomenon will be considered in further detail in the Discussion.

5. *Temporary parabiosis between previously chilled pupae and abdomens of male Cecropia moths*

How long must a pupa be joined to a male Cecropia abdomen before it will give a positive test for juvenile hormone? This question was studied in six preparations in which the initial parabiosis was disassembled after a certain time and the adult abdomen grafted to a fresh pupa.

The results were as follows: When the initial parabiosis was for only one day, the pupa developed into a normal moth, whereas the second pupal partner developed into a mixture of pupa and adult. But when the first parabiosis was extended to five days, then both the first and the second pupal partners gave positive tests for juvenile hormone. A transient blood connection with the adult is therefore sufficient.

In additional experiments testing this point it appears that the parabiosis must continue until the pupal partner actually initiates its adult development; in the above-mentioned experiments this occurred on the fourth day. If the pupa requires more than four days to initiate its development, then the parabiosis must be continued until it does so.

6. *Effects of allatectomy*

The results show that abdomens of male Cecropia and Cynthia moths are somehow able to cause a positive test for juvenile hormone when joined to pupal partners. Since the adult corpora allata are cephalic structures, the moth abdomen did not appear to be a reasonable candidate for the secretion of juvenile hormone. Attention therefore centered on the corpora allata in the pupal partner. Despite the fact that pupal corpora allata are known to be inactive (Williams, 1961), it seemed possible that they might be "turned on" by some influence arising in the moth abdomen. In order to test this possibility, the series of experiments, summarized in Table IV, was carried out.

Corpora allata were excised from four previously chilled Cecropia pupae. Then, each allatectomized pupa was joined to a headless male Cecropia moth. Though the preparations now contained no corpora allata, a positive test for juvenile hormone was obtained in each case. This result leaves no reasonable doubt that the source of the juvenile hormone was the adult abdomen itself. This conclusion was tested as follows:

The corpora allata and corpora cardiaca were excised from six chilled male Cecropia pupae which were then placed at 25° C. and allowed to develop into adult moths. Three of these allatectomized moths were beheaded and joined to previously chilled pupae; in the other three, the moth abdomens were excised and used in the parabiosis. As recorded in Table IV, all six preparations now gave negative tests for juvenile hormone. This shows that the accumulation of juvenile hormone by the abdomen is dependent on its synthesis and secretion by the corpora allata in the head.

In a further test, the corpora allata and corpora cardiaca were excised from two previously chilled male Cecropia pupae and two pairs of "loose" pupal corpora

Table IV

Parabiosis between previously chilled pupae and male Cecropia moths; either the pupal or adult partner had been allatectomized

Adult component	Pupal partner	Number of experiments	Effects on pupal partner
Headless ♂ Cecropia	Allatectomized ♀ Cecropia	4	Developed into moths retaining many pupal characters
Headless ♂ Cecropia (allatectomized in pupal stage)	♂ Cecropia	3	Formed normal moths
♂ Cecropia abdomen (allatectomized in pupal stage)	♂ Cecropia	3	Formed normal moths
♂ Cecropia abdomen (allatectomized in pupal stage and two pairs of pupal corpora allata re-implanted into head)	♂ Cecropia	2	Developed into moths retaining many pupal characters

allata-cardiaca were re-implanted into the head. When the moths emerged, their abdomens were joined to chilled pupae. The latter gave positive pupal assays for juvenile hormone (Table IV). This indicates that implanted corpora allata can substitute for the animal's own corpora allata in the secretion of juvenile hormone.

7. *Elution of juvenile hormone from abdominal tissues of male Cecropia moths*

On the basis of the experiments just considered, it seems necessary to conclude that the juvenile hormone is secreted by the corpora allata in the head of the adult moth and that, in the case of male Cecropia and Cynthia moths, the abdomen is somehow able to bind and accumulate substantial amounts of hormone. We are led to the prediction that the abdomen of the male Cecropia moth must contain a depot of juvenile hormone. A direct test of this inference was made as follows:

The abdomens of several male Cecropia moths were dissected in Ringer's solution. Various tissues and organs were removed, rinsed, and tested by implantation under a plastic window at the tip of the abdomen of previously chilled pupae. The results, summarized in Table V, show that positive assays for juvenile hormone

TABLE V

*Tests for juvenile hormone in abdominal tissues and organs of adult male Cecropia moths**

Adult tissue	Number of preparations	Results of tests
Fragment of fat-body	3	2 positive; 1 negative
Abdominal ganglia and connectives	4	1 positive; 3 negative
Vas deferens	5	1 positive; 4 negative
Testes	2	Negative
Gut	1	Negative
Solution of egg yolk from adult female	3	Negative

* The tissues or organs were implanted into the tip of the abdomen of previously chilled Cecropia or Polyphemus pupae. The test was scored as positive when the pupa formed a moth retaining pupal characters.

were obtained in certain cases after the implantation of adult fat-body, abdominal nerve cords, or vas deferens. The positive test in each case was a minimal reaction in that pupal cuticle was re-formed only under the abdominal window at the site of injury (Williams, 1961). A number of other adult organs, including a solution of yolk obtained from unfertilized Cecropia eggs, gave negative tests.

DISCUSSION

1. *The sequestering of juvenile hormone*

The experimental results reveal the surprising fact that the abdomens of male Cecropia and Cynthia moths contain a cache of juvenile hormone. The hormone is found in the most prominent tissue of the abdomen—the fat-body—and also in at least two other tissues, the nerve cord and the vas deferens. Moreover, the hormone can be eluted from these tissues when the latter are either implanted or joined to a test pupa by way of the hemolymph. This shows that the sequestered hormone is in some sort of dynamic equilibrium with the circulating blood.

Though the hormone accumulates in the abdomen, it is synthesized by the corpora allata in the head (Fig. 5). So, if the corpora allata are excised prior to the final week of adult development, then, as diagrammed in Figure 6, no hormone accumulates in the abdomen.

It will be recalled that the corpora allata are inactive in the pupa and that this inactivity persists during two-thirds of adult development (Williams, 1961). Then, on or about the fourteenth day of adult development, the corpora allata recover the activity they had lost months earlier at the time of pupation. Yet, strange to say, the juvenile hormone has no known function in the adults of these short-lived Lepidoptera. Allatectomized Cecropia pupae develop into normal male and female moths which, when cross-mated, give rise to fertile eggs (Williams, 1959).

Despite persistent uncertainties as to the role of juvenile hormone in these adult insects, we can confidently say that the hormone is sequestered in the abdominal

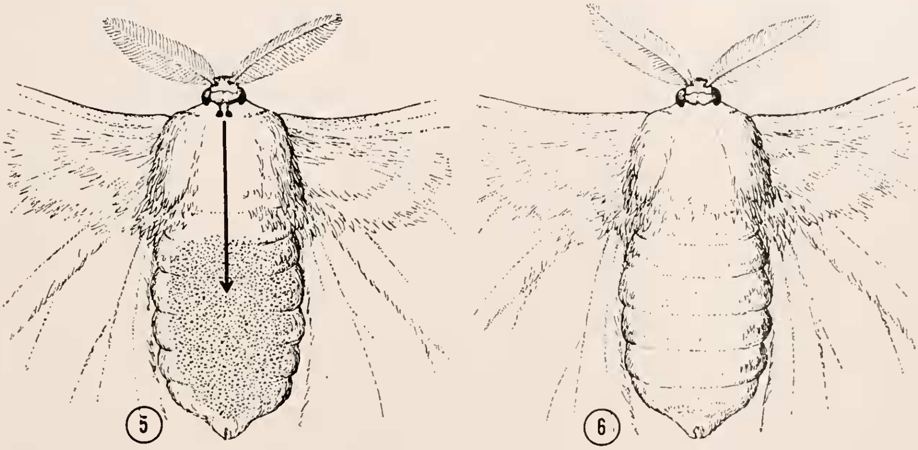

FIGURE 5. As indicated by the stippled area, the juvenile hormone which accumulates in the abdomen of the male Cecropia moth is synthesized and secreted by the corpora allata in the head.

FIGURE 6. If the corpora allata are removed from the head, then no hormone accumulates in the abdomen. (Figures 5 and 6 are used with the permission of the *Scientific American*.)

tissues of male Cecropia and Cynthia moths. This process presumably begins after the corpora allata regain their activity during the final week of adult development, and continues during the entire week of adult life (Williams, 1961). The abdomen, in effect, serves as an extraction chamber which, over a period of about two weeks, binds and accumulates the hormone which is continuously secreted by the corpora allata.

2. *The species- and sex-specific accumulation of juvenile hormone*

The accumulation of hormone takes place in male Cecropia and Cynthia moths, but only rarely in females. Yet the corpora allata of female moths are just as active in secreting juvenile hormone as are those of males (Williams, 1959). Moreover, the accumulation of juvenile hormone occurs in neither male nor female

Pernyi or Polyphemus moths despite the fact that the adult corpora allata of these insects are extremely active in the secretion of juvenile hormone (Williams, 1959).

For these several reasons it is amply evident that those moths which fail to accumulate the hormone must dispose of it in some manner, presumably by converting it into inactive products. Whatever this inactivating mechanism may be, it is obviously curtailed in the case of male Cecropia and Cynthia moths.

3. *The induced molting of adult moths*

Among the pterygote insects only the Ephemeroptera are known to molt as adults—a phenomenon which has recently been subjected to detailed study by Taylor and Richards (1963). Yet, as demonstrated in the present study, one can often cause an adult moth to molt by joining it in parabiosis with a pupal partner.

The molt consisted of a detachment and retraction of the epidermis from the adult cuticle and the secretion of a new cuticle; in no case did the moth make any effort to escape from the exuviae. The molting of the adult began in synchrony with that of the pupal partner. When the latter was a diapausing pupa and failed to initiate development (Table I), then no trace of molting occurred in the adult. This shows that the molting of the moth was a response to the ecdyson secreted by the pupal prothoracic glands and conveyed to the moth in the pupal blood.

Manifestly, the cells and tissues of the moth retain the potential for further development and molting when supplied with ecdyson. The failure of adult insects to molt is therefore directly attributable to the breakdown of their prothoracic glands and the consequent lack of ecdyson.

In the parabiotic preparations molting occurred in only about one-third of the moths. This capricious result was apparently due to uncontrollable variations in the circulation of blood between the pupal and adult partners. The blood connection which one can establish at the anterior end of a headless moth is, perforce, of small diameter. And when an adult abdomen was used, the blood flow from the pupa was commonly impaired by the herniation into the pupa of the moth ovaries and the fluid-filled rectal sac. In many of the adult partners these circumstances apparently precluded the build-up of a threshold titer of the ecdyson.

In most preparations the molting of the moth was incomplete and one could not remove the loose cuticle because of its persistent attachments to the spiracles and genitalia. However, in occasional preparations the molt was complete and the old cuticle could be easily peeled from the abdomen.

A preparation of this type is shown in Figure 7. The entire adult cuticle has been molted, including the intricate cuticle of the male genitalia. The new cuticle preserves the smooth texture and pale tan pigmentation of normal adult cuticle, the only striking difference being the virtual absence of scales or hairs. The naked character of the new adult cuticle was especially prominent in Cecropia and was apparently due to the depletion of "scale mother cells" which during the formation of the first adult cuticle were transformed into lifeless scales and sockets (Henke, 1946). However, in the case of Polyphemus or Pernyi abdomens, the new adult cuticle commonly showed substantial clusters of scales along the dorsum of the abdominal segments; this apparently signifies the presence in these species of a reserve supply of scale and socket "stem cells."

In all of the preparations that molted, a new adult cuticle formed, irrespective of whether the molt occurred in the presence or absence of juvenile hormone. The new cuticle showed no trace of the reappearance of pupal characteristics even when the molt took place in the presence of sufficient juvenile hormone to cause the pupal partner to form a second pupal stage. This result is therefore different from that reported for *Rhodnius* where, according to Wigglesworth (1940, 1958), a partial

FIGURE 7. The abdomen of a male Cecropia moth was joined with a previously chilled pupa of the Cecropia silkworm. Here, after four weeks at 25° C., the pupa has transformed into a moth, preserving numerous pupal characters. Meanwhile, the moth abdomen has fully molted its adult cuticle and formed a new cuticle of adult type, except for the absence of scales and hairs.

"reversal of metamorphosis" occurs when an adult is caused to molt in the presence of juvenile hormone.

4. *Prolongation of adult life*

In all the species used in the present study the moths are unable to feed because of the absence of functional mouth parts. Therefore, they rarely survive at room temperature for more than a week. And yet, when beheaded and joined to diapausing pupae, these same moths routinely survived for many weeks, during which time one could elicit leg movements and the flapping of the wings. Even the most moribund moth, within a few hours of death, reacted to parabiosis with renewed vitality and longevity.

Manifestly, the moths are sustained by the fluids and substrates which are continuously transfused *via* the pupal blood. This shows that the moths normally die of desiccation and nutritional deficiencies, and that the adult cells and tissues are capable of a far longer life-span than they can ordinarily display.

5. *Sexual reflexes of headless moths and isolated abdomens*

Many species of insects show exaggerated sexual reflexes following decapitation —an effect attributable to a release from the normal inhibitory influences of the cephalic ganglia (Roeder *et al.*, 1960). Effects of this sort were particularly prominent in parabiotic preparations where the decapitated individual survived for up to ten weeks.

The effects were most spectacular in the headless males or isolated male abdomens of Pernyi and Cecropia. Here, immediately after recovery from anesthesia, the individual initiated lively motion of both its abdomen and claspers, precisely as in normal mating. This behavior usually continued for several days, and was accompanied by the discharge of one or more spermatophores from the extended aedeagus. This curious behavior on the part of the headless male moth or male abdomen was far less conspicuous in Orizaba and totally lacking in Polyphemus.

In general, the effects of decapitation were not as prominent in the case of female moths. One generally observed a great increase in the tone of the intersegmental muscles and a downward flexion of the tip of the abdomen as in normal egg-laying. However, no eggs were actually oviposited, even though the abdomen was full of them.

6. *The mimicking of brain hormone by juvenile hormone*

According to experiments reported previously (Williams, 1959), the implantation of active corpora allata can cause the initiation of development in a certain proportion of brainless diapausing pupae. On further analysis, the conclusion was reached that under certain conditions the juvenile hormone can activate the prothoracic glands and in this sense mimic the function of the brain hormone.

These earlier results are strikingly reminiscent of those recorded in Table I. Here, 4 of 9 diapausing Cecropia pupae were caused to initiate development by joining them in parabiosis with headless male Cecropia moths. The net effect in this case, as in the experiments reported four years ago, was the termination of diapause and the production of pupal-adult monstrosities.

The stimulation of development in the pupal partner, as noted in Table I, was observed when diapausing pupae were joined to male Cecropia moths which, as we have seen, contain a rich depot of juvenile hormone. In the absence of sequestered hormone, headless female Cecropia moths, as well as headless Polyphemus and Pernyi of both sexes, gave negative tests in all cases.

Consequently, the experiments summarized in Table I are additional evidence that the juvenile hormone can mimic the brain hormone in its ability to turn on the prothoracic glands.

Summary

1. Pupae joined in parabiosis with headless male Cecropia moths behave as if they have received an injection of juvenile hormone. They develop, not into

normal moths, but into creatures which show a mixture of pupal and adult characters.

2. By diverse experiments it was possible to show that the juvenile hormone comes from the moth abdomen and that the abdominal tissues of male Cecropia moths contain a rich depot of juvenile hormone.

3. In the moth, itself, the hormone is synthesized by the corpora allata in the head and is progressively bound and sequestered by the abdominal tissues. If the corpora allata are removed from the head, then no hormone accumulates in the abdomen.

4. The accumulation of juvenile hormone in the abdominal tissues occurs in male Cecropia and Cynthia moths, but not in females. In the case of certain related species of saturniid moths (Polyphemus, Pernyi and Orizaba), neither sex is ordinarily able to accumulate the hormone, despite the fact that they have very active corpora allata.

5. The failure to accumulate the hormone points to some unknown means for its inactivation; these agencies are evidently curtailed or by-passed in the case of male Cecropia and Cynthia moths which accumulate large amounts of hormone.

6. Adult moths are frequently caused to molt when joined to non-diapausing pupae and thereby supplied with ecdyson. A new adult cuticle forms which is deficient in scales and hairs. Adults molting in the presence of high concentrations of juvenile hormone show no reappearance of pupal characters or any sign of a "reversal of metamorphosis."

7. When the adult tissues are continuously perfused with pupal blood, the life-span of the moths is greatly prolonged. This shows that, in the absence of functional mouthparts, the moth normally dies of desiccation and starvation rather than from the intrinsic biological death of the tissues themselves.

8. In experiments involving the parabiosis of diapausing pupae with moths containing a depot of juvenile hormone, additional evidence was obtained that juvenile hormone can turn on the prothoracic glands and, in this sense, mimic the brain hormone.

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